

various methods well known in the art. Transformation may rely on any known method for the insertion of nucleic acid sequences into a prokaryotic or eukaryotic host cell, including microorganism-mediated transformation, viral infection, whiskers, electroporation, microinjection, polyethylene glycol-treatment, heat shock, lipofection, and particle bombardment.

**[0095]** Transgenic organism comprises at least one cell in which an exogenous nucleic acid has been stably integrated. A transgenic organism according to the invention is for instance a bacterial, or eukaryotic, such as a yeast, host cell or host organism. The bacterium can be chosen from the group comprising Gram-negative and Gram-positive bacteria, such as, but not limited to, *Escherichia* spp. (e.g. *E. coli*), *Bacillus* spp. (e.g. *B. thuringiensis*), *Rhizobium* spp., *Lactobacillus* spp., *Lactococcus* spp., etc. The yeast can be chosen from the group comprising *Saccharomyces* spp., etc.

**[0096]** Variant: a “variant,” as used herein, is understood to mean a nucleotide sequence that deviates from the standard, or given, nucleotide or amino acid sequence of a particular gene or protein. The terms, “isoform,” “isotype,” and “analog” also refer to “variant” forms of a nucleotide sequence. “Variant” may also refer to a “shuffled gene” such as those described in Maxygen-assigned patents.

**[0097]** It is understood that the present invention is not limited to the particular methodology, protocols, vectors, and reagents, etc., described herein, as these may vary. It is also to be understood that the terminology used herein is used for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention. It must be noted that as used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to “a gene” is a reference to one or more genes and includes equivalents thereof known to those skilled in the art and so forth.

**[0098]** I. Target Pests

**[0099]** The present invention provides methodology and constructs for controlling pest infestations by administering, or otherwise exposing, to a pest a target coding sequence that post-transcriptionally represses or inhibits a requisite biological function in the pest. As used herein, the term “pest” refers to insects, arachnids, crustaceans, fungi, bacteria, viruses, nematodes, flatworms, roundworms, pinworms, hookworms, tapeworms, trypanosomes, schistosomes, botflies, fleas, ticks, mites, and lice and the like that are pervasive in the human environment. A pest may ingest or contact one or more cells, tissues, or products produced by an organism transformed with a double stranded gene suppression agent, as well as a surface or material treated with a double stranded gene suppression agent.

**[0100]** A “pest resistance” trait is a characteristic of a transgenic host that causes the host to be resistant to attack from a pest that typically inflicts damage to the host. Such pest resistance can arise from a natural mutation or more typically from incorporation of recombinant DNA that confers pest resistance. To impart pest resistance to a transgenic host, a recombinant DNA can, for example, be transcribed into a RNA molecule that forms a dsRNA molecule within the tissues or fluids of the recombinant host. The dsRNA molecule is comprised in part of a segment of RNA that is identical to a corresponding RNA segment encoded from a DNA sequence within a pest that prefers to feed on the recombinant host. Expression of the gene within the target pest is sup-

pressed by the dsRNA, and the suppression of expression of the gene in the target pest results in the host being pest resistant.

**[0101]** Suitable pests include any organism that causes damage to another organism. The invention contemplates insect, nematode, and fungal pests in particular.

**[0102]** Insect as used herein can be any insect, meaning any organism belonging to the Kingdom Animals, more specific to the Phylum Arthropoda, and to the Class Insecta or the Class Arachnida. The methods of the invention are applicable to all insects and that are susceptible to gene silencing by RNA interference and that are capable of internalising double-stranded RNA from their immediate environment.

**[0103]** In one embodiment of the invention, the insect may belong to the following orders: Acari, Araneae, Anoplura, Coleoptera, Collembola, Dermaptera, Dictyoptera, Diplura, Diptera, Embioptera, Ephemeroptera, Grylloblatodea, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mallophaga, Mecoptera, Neuroptera, Odonata, Orthoptera, Phasmida, Plecoptera, Protura, Psocoptera, Siphonaptera, Siphunculata, Thysanura, Strepsiptera, Thysanoptera, Trichoptera, and Zoraptera.

**[0104]** In preferred, but non-limiting, embodiments and methods of the invention the insect is chosen from the group consisting of:

**[0105]** (1) an insect which is a plant pest, such as but not limited to *Nilaparvata* spp. (e.g. *N. lugens* (brown planthopper)); *Laodelphax* spp. (e.g. *L. striatellus* (small brown planthopper)); *Nephotettix* spp. (e.g. *N. virescens* or *N. cincticeps* (green leafhopper), or *N. nigropictus* (rice leafhopper)); *Sogatella* spp. (e.g. *S. furcifera* (white-backed planthopper)); *Blissus* spp. (e.g. *B. leucopterus leucopterus* (chinch bug)); *Scotinophora* spp. (e.g. *S. vermidulate* (rice blackbug)); *Acrosternum* spp. (e.g. *A. hilare* (green stink bug)); *Parnara* spp. (e.g. *P. guttata* (rice skipper)); *Chilo* spp. (e.g. *C. suppressalis* (rice striped stem borer), *C. auricilius* (gold-fringed stem borer), or *C. polychrysus* (dark-headed stem borer)); *Chilotranea* spp. (e.g. *C. polychrysa* (rice stalk borer)); *Sesamia* spp. (e.g. *S. inferens* (pink rice borer)); *Tryporyza* spp. (e.g. *T. innotata* (white rice borer), or *T. incertulas* (yellow rice borer)); *Cnaphalocrocis* spp. (e.g. *C. medinalis* (rice leafroller)); *Agromyza* spp. (e.g. *A. oryzae* (leafminer), or *A. parvicornis* (corn blot leafminer)); *Diatraea* spp. (e.g. *D. saccharalis* (sugarcane borer), or *D. grandiosella* (southwestern corn borer)); *Narnaga* spp. (e.g. *N. aenescens* (green rice caterpillar)); *Xanthodes* spp. (e.g. *X. transversa* (green caterpillar)); *Spodoptera* spp. (e.g. *S. frugiperda* (fall armyworm), *S. exigua* (beet armyworm), *S. littoralis* (climbing cutworm) or *S. praefica* (western yellowstriped armyworm)); *Mythimna* spp. (e.g. *Mythimna (Pseudaletia) seperata* (armyworm)); *Helicoverpa* spp. (e.g. *H. zea* (corn earworm)); *Colaspis* spp. (e.g. *C. brunnea* (grape colaspis)); *Lissorhoptrus* spp. (e.g. *L. oryzophilus* (rice water weevil)); *Echinocnemus* spp. (e.g. *E. squamos* (rice plant weevil)); *Diodesia* spp. (e.g. *D. armigera* (rice hispa)); *Oulema* spp. (e.g. *O. oryzae* (leaf beetle)); *Sitophilus* spp. (e.g. *S. oryzae* (rice weevil)); *Pachydiplosis* spp. (e.g. *P. oryzae* (rice gall midge)); *Hydrellia* spp. (e.g. *H. griseola* (small rice leafminer), or *H. sasakii* (rice stem maggot)); *Chlorops* spp. (e.g. *C. oryzae* (stem maggot)); *Ostrinia* spp. (e.g. *O. nubilalis* (European corn borer)); *Agrotis* spp. (e.g. *A. ipsilon* (black cutworm)); *Elasmopalpus* spp. (e.g. *E. lignosellus* (lesser cornstalk borer)); *Melanotus* spp. (wireworms); *Cyclocephala* spp. (e.g. *C. borealis* (northern masked chafer), or *C. immaculata*